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Hormonal Regulation of Liver Metallothionein Zinc: Independent and Synergistic Action of Glucagon and Glucocorticoids[1] (41155)

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Abstract. Recent evidence suggests glucocorticoids alter hepatic zinc metabolism. The induction of metallothionein synthesis seems to be one of the key regulatory steps involved. The experiments reported here were designed to examine the influence of glucagon on liver metallothionein-bound zinc. Glucagon (1.0 mg/kg) and/or dexamethasone (2.0 mg/kg) was administered to 175–225 g male rats. An independent, synergistic effect of glucagon which increased the binding of zinc to liver metallothionein induced by glucocorticoids was identified. The glucagon response was short lived with a maximum 7 hr after administration and a return to initial levels 7 hr thereafter. A maximal increase in metallothionein-bound zinc in response to dexamethasone, a synthetic glucocorticoid, increased steadily to 21 hr after administration. Administration of both the glucocorticoid and glucagon resulted in a significant, but short-lived, augmentation of the action of either hormone alone. Actinomycin D blocked the dexamethasone effect but had no effect on glucagon action, suggesting the latter does not involve *de novo* RNA synthesis. These results suggest that changes in hepatic metallothionein-bound zinc involve synergistic effects of glucagon and glucocorticoids which act through two separate, yet related mechanisms. These findings expand our view of the widespread role of glucagon and glucocorticoids in liver function and demonstrate that the regulation of hepatic zinc metabolism has multihormonal components.

Zinc has a wide variety of functions in mammals, including roles which influence metalloenzyme activity and stabilization of certain macromolecules (1–3). The liver plays a central role in the metabolism of this essential nutrient (4–6). Despite modest changes in the dietary zinc supply, animals are normally capable of homeostatically regulating liver zinc levels (7–11). Alternatively, periods of stress, trauma, infection, and starvation produce dramatic yet predictable changes in liver and plasma zinc (12–15). The hormonal basis of the regulation of the zinc-related changes in liver, although relevant to a variety of body defense mechanisms, remains obscure.

Recent evidence from our laboratory and others indicate that glucocorticoids are involved in regulating certain aspects of hepatic zinc metabolism. Specifically glucocorticoids induce the formation of metallothionein, a zinc binding protein in hepatocytes (7, 16). This metalloprotein has been shown to be closely linked to zinc accumulation and intracellular redistribution (18, 19). The increased synthesis of metallothionein explains how the liver accumulates additional zinc during periods of stress and affects a reduction in plasma zinc. The teleological significance of stress-induced metallothionein is not understood at present. Since glucocorticoids are not the only hormones that play a major role in controlling liver function, it seemed plausible that the regulation of liver metallothionein might have multihormonal components. We now present evidence to support the regulatory action of glucagon on the induction of liver metallothionein zinc *in vivo*.

Materials and Methods. Male CD strain rats (175–225 g, Charles River Breeding Lab) were used. Adrenalectomies were performed under sodium pentobarbitol anesthesia (60 mg/kg; ip) by the paravertebral dorsal approach, at least 6 days prior to experimentation. All rats were maintained in suspended stainless-steel, wire-bottom

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cages and had free access to tap water and a standard diet (Ralston-Purina Co.) until they were sacrificed. Adrenalectomized rats were provided with a 0.9% NaCl solution instead of tap H_2O. Dexamethasone (sodium phosphate salt) was dissolved in 0.9% NaCl and injected (2.0 mg/kg; ip) at the indicated times prior to sacrifice. Control rats received equivalent doses of 0.9% NaCl. All rats were killed by decapitation between 1200 and 1400 hr to minimize variation due to circadian rhythms. Livers were homogenized (1:1 w/v) in ice-cold, 0.25 *M* sucrose (in 10 m*M* Tris–acetate; pH 8.6) with a Polytron homogenizing apparatus (Brinkmann Instruments). The homogenate was centrifuged at 42,000*g* for 12 min at 4°. The supernatant was removed and recentrifuged at 166,000*g* for 1 hr at 4° to yield a final supernatant solution (cytosol). Immediately thereafter, 4.0 ml of the cytosol was applied to a 2.5 × 60 cm column of Sephadex G-75, equilibrated with 10 m*M* Tris–acetate (pH 8.6) at 4°. Flow rate (28 ml/hr) was maintained with a peristaltic pump. Zinc metallothionein routinely eluted with a V/V_0 value of 1.9. This procedure provides a major purification of the protein and has been used previously as an assay for metallothionein (6–10, 20). Zinc content of the fractions (3.0 ml) was measured by atomic absorption spectrophotometry. These assay columns were extremely reproducible and routinely the zinc content of 14 fractions were totaled to derive the metallothionein-bound zinc content. Data were analyzed by standard analysis of variance procedures.

Results and Discussion. A definitive influence of dexamethasone, a synthetic glucocorticoid, on the amount of metallothionein-bound zinc in the cytosol fraction (166,000*g* supernatant) of livers from either intact or adrenalectomized rats can be demonstrated. The hormone was administered (2.0 mg/kg) at various times before metallothionein was isolated. As summarized in Table I, the glucocorticoid significantly increased the amount of zinc bound in the liver cytoplasm as metallothionein. In the intact rats a maximal level was reached at about 14 hr. In contrast, when this hormone was administered to adrenalectomized rats the initial response was of significantly greater magnitude. This differential response demonstrates that the induction of metallothionein by dexamethasone occurs via regulation of a glucocorticoid sensitive process rather than via a nonspecific route.

Subsequently, we have found that exposure to a cold environment (4°) was also capable of increasing liver metallothionein zinc in adrenalectomized rats. Since this response to a stress could take place in the absence of glucocorticoids, we considered the possibility that another hormonal mechanism could regulate liver metallothionein zinc levels. We began a series of experiments with intact rats to examine the influence of glucagon, alone or in combination with dexamethasone, on liver metallothionein-bound zinc. Glucagon administration (1.0 mg/kg) produced a brief, but significant, rise in relative content of zinc associated with metallothionein (Fig. 1). However, administration of dexamethasone (2.0 mg/kg), 1 hr prior to glucagon, produced a significant augmentation of the effect between 4 and 8 hr after the hormones were administered. These data

TABLE I. ZINC CONTENT OF LIVER METALLOTHIONEIN IN NORMAL AND ADRENALECTOMIZED RATS FOLLOWING DEXAMETHASONE ADMINISTRATION

	μg Zn as metallothionein/ml liver cytosol				
Hours after dexamethasone	0	3.5	7	14	21
Nonadrenalectomized	1.1 ± 0.1	0.8 ± 0.1	1.7 ± 0.2[a]	2.3 ± 0.1[a]	3.0 ± 0.7[a]
Adrenalectomized	0.9 ± 0.1	1.4 ± 0.2[a,b]	4.1 ± 0.4[a,b]	3.6 ± 0.3[a]	3.6 ± 0.4[a]

Note. Data are based upon four animals/group and expressed as μg zinc in metallothionein/ml of cytosol. Means ± SEM are shown.

[a] Significantly different from 0-hr values within each animal group ($P < 0.05$).

[b] Significantly different from nonadrenalectomized group ($P < 0.05$).

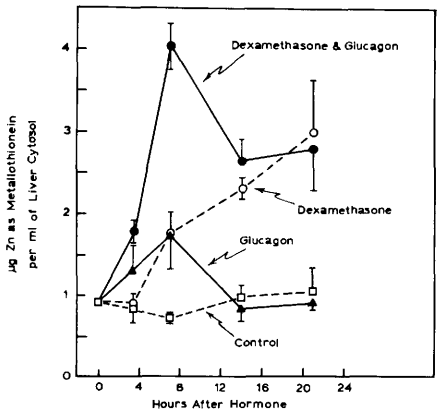

FIG. 1. Liver metallothionein zinc following glucagon and/or dexamethasone administration to nonadrenalectomized rats. Dexamethasone (2.0 mg/kg) or saline (0.9% NaCl) was administered 1 hr prior to glucagon (1.0 mg/kg) or saline. Data (four animals per group) are expressed micrograms of Zn as metallothionein per milliliter of cytosol. Abscissa refers to time after the second injection (either glucagon or saline). Means ± SEM are shown. These group means were significantly different ($P < 0.05$) from the control group mean: all time points of dexamethasone + glucagon; 7-, 14-, and 21-hr points of dexamethasone alone; 7-hr point of glucagon alone. These group means were significantly different ($P < 0.01$); dexamethasone alone and dexamethasone + glucagon vs glucagon alone at 14- and 21-hr and 7-, 14-, and 21-hr points, respectively; dexamethasone + glucagon vs all groups at 7 hr.

suggest that although glucagon is able to increase metallothionein-bound zinc above a basal level, glucocorticoids are required for maximal, long-lasting changes in bound zinc. Preliminary evidence suggested glucagon had no influence on serum zinc concentrations as we demonstrated 14–21 hr after dexamethasone treatment (21). Neither glucagon nor dexamethasone produced measurable changes in the total liver zinc content.

Previously we found induction of liver metallothionein by dexamethasone involved changes in its rate of synthesis and the polyribosomal pool of metallothionein mRNA (21). The lag in appearance of the protein in response to the hormone was explained on the basis of *de novo* metallothionein mRNA biosynthesis (21–23). In order to investigate the possible mechanism(s) by which glucagon alone and combined with dexamethasone affects metallothionein bound zinc, we injected actinomycin D 4 hr prior to administration of the hormones. The level of metallothionein zinc 7 hr after glucagon injection was not significantly changed by this inhibitor of mRNA synthesis (Table II). However, actinomycin D blocked the combined action of dexamethasone and glucagon. These data suggest that the increase in metallothionein zinc caused by these two hormones is mediated through different mechanisms which are capable of interacting to produce a synergistic effect early in the induction sequence. The effect of dexamethasone requires *de novo* RNA synthesis whereas that of glucagon does not. At the present time, the origin of the additional zinc bound to the protein is not clear. It is generally believed that metallothionein sequesters intracellular zinc (18, 19). Since the turnover of the protein is too long (23) to explain the glucagon-induced changes in metallothionein zinc and enhanced RNA synthesis does not appear to be involved, it is likely the metal has been acquired by existing metallothionein polypeptide chains as the result of enhanced transport of extracellular zinc or acquisition from intracellular zinc pools.

The permissive action of glucocorticoids upon the action of various other hormones is well documented. Specifically, Friedman *et al.* and Exton *et al.* (24, 25) have demonstrated that the failure of glucagon to activate hepatic gluconeogenesis in adrenalectomized rats can be reversed by glucocorticoid treatment *in vivo* and *in*

TABLE II. INFLUENCE OF ACTINOMYCIN D ON LIVER METALLOTHIONEIN ZINC FOLLOWING DEXAMETHASONE AND/OR GLUCAGON

	μg Zn as metallothionein/ml liver cytosol		
	Saline	Glucagon	Glucagon + dexamethasone
Control	0.7 ± 0.1	1.7 ± 0.4†	4.0 ± 0.3†
Actinomycin D	0.5 ± 0.1	1.5 ± 0.3†	1.4 ± 0.1†·*

Note. Glucagon (1.0 mg/kg), dexamethasone (2.0 mg/kg), and actinomycin D (0.8 mg/kg) were administered 7, 8, and 12 hr, respectively, prior to sacrifice. Four animals were used per group. Mean ± SEM are shown. * Indicates difference from control group is statistically significant while † indicates differences from saline injected groups are statistically significant, both at $P < 0.05$.

vitro. Although our data show that glucagon by itself is capable of increasing liver metallothionein zinc very rapidly, the duration of its action is relatively short compared to the combined dexamethasone–glucagon administration. Moreover, the synergistic, but independent actions of glucagon and glucocorticoids on metallothionein are closely analogous to the regulatory influences these hormones have on amino acid transport in liver cells (26, 27). These hormones also have profound effects on numerous enzymes, e.g., tyrosine aminotransferase (28).

Although most of the interest in metallothionein has centered on its relationship to metal binding, its regulation at the hormonal level should be viewed in a broad context in which both the protein and its zinc content are integrated into other aspects of liver function. This new observation, demonstrating a role for glucagon in augmenting metallothionein synthesis and concomitant zinc binding, could provide a valuable model system for the investigation of multicomponent hormone action on liver cells. Moreover, these results suggest that changes in hepatic metallothionein bound zinc observed during starvation, stress, and trauma involve synergistic effects of glucagon and glucocorticoids.

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